TOREMIFENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Toremifene

Chem. Abstr. Serv. Reg. No.: 89778-26-7

Deleted CAS Reg. No.: 98644-21-4

Chem. Abstr. Name: (*Z*)-2-[4-(4-Chloro-1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine

IUPAC Systematic Name: 2-[*para*-[(*Z*)-4-Chloro-1,2-diphenyl-1-butenyl]phenoxy]-*N*,*N*-dimethylethylamine

Synonyms: (*Z*)-4-Chloro-1,2-diphenyl-1-(4-(2-(*N*,*N*-dimethylamino)ethoxy)phenyl)-1-butene; *Z*-toremifene; toremifene base

Toremifene citrate

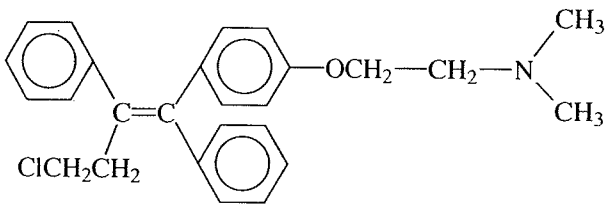
Chem. Abstr. Serv. Reg. No.: 89778-27-8

Chem. Abstr. Name: (*Z*)-2-[4-(4-Chloro-1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)

IUPAC Systematic Name: 2-[*para*-[(*Z*)-4-Chloro-1,2-diphenyl-1-butenyl]phenoxy]-*N*,*N*-dimethylethylamine citrate (1:1)

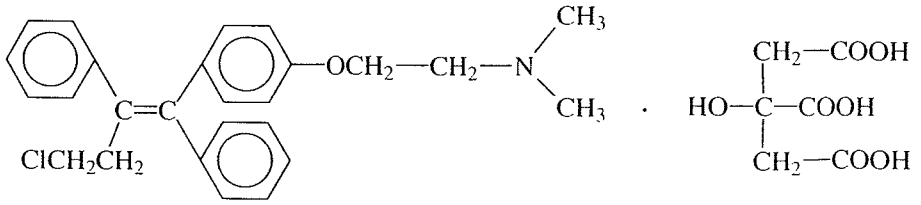
Synonyms: (*Z*)-4-Chloro-1,2-diphenyl-1-[4-[2-(*N*,*N*-dimethylamino)ethoxy]phenyl]-1-butene citrate (1:1)

1.1.2 *Structural and molecular formulae and relative molecular mass*



Toremifene

$C_{26}H_{28}ClNO$ Relative molecular mass: 405.87



Toremifene citrate

$C_{26}H_{28}ClNO.C_6H_8O_7$ Relative molecular mass: 598.10

1.1.3 *Chemical and physical properties of the pure substances*

Toremifene

Melting-point: 108–110 °C (Budavari, 1995)

Toremifene citrate

(*a*) *Description*: White crystals (Orion, 1996)

(*b*) *Melting-point*: 160–162 °C (Budavari, 1995)

(*c*) *Spectroscopy*: Ultraviolet, infrared, nuclear magnetic resonance and mass spectrometric data have been reported (Orion, 1996)

(*d*) *Solubility*: Sparingly soluble in methanol; slightly soluble in ethanol; very slightly soluble in water (0.44 mg/mL at 37 °C), acetone and chloroform; practically insoluble in octanol and diethyl ether (Budavari, 1995; Orion, 1996)

(*e*) *Stability*: Sensitive to ultraviolet light (Orion, 1996)

(*f*) *Dissociation constant*: pK_a, ~ 8.0 (Orion, 1996)

(*g*) *Octanol/water partition coefficient (P)*: log P, 3.3 (Orion, 1996)

1.1.4 *Technical products and impurities*

Toremifene in pharmaceutical preparations is invariably present as its citrate salt.

Toremifene citrate is available as an 88.5-mg tablet (equivalent to 60 mg toremifene base), which may also contain maize starch, lactose, polyvinylpyrrolidone (povidone), sodium starch glycolate, magnesium stearate, microcrystalline cellulose and colloidal anhydrous silica (Orion, 1996).

The *E*-isomer may be present as a minor impurity (≤ 0.3%) (Orion, 1996).

Trade names and designations for toremifene citrate and its pharmaceutical preparations include: FC 1157a; Fareston; NK 622.

1.1.5 *Analysis*

Toremifene and its metabolites can be analysed in biological fluids by liquid chromatography–atmospheric pressure ionization mass spectrometry (Watanabe *et al.*, 1989) and high-performance liquid chromatography (Holleran *et al.*, 1987; Hasan *et al.*, 1990; Webster *et al.*, 1991; Berthou & Dréano, 1993; Lim *et al.*, 1994).

1.2 Production and use

1.2.1 *Production*

Toremifene was first synthesized in 1981. Toremifene was first marketed commercially in 1990; production in 1995 was about 100 kg (Orion, 1996).

The synthesis of toremifene citrate consists of four process phases. In the first phase, 4-hydroxybenzophenone is O-alkylated with 2-chloroethyl dimethylamine yielding the first intermediate [4-(2-dimethylaminoethoxy)phenyl]phenylmethanone. This intermediate is condensed with a complex formed of cinnamaldehyde and lithium aluminium hydride and the second intermediate 1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbutane-1,4-diol is obtained. This intermediate is treated with thionyl chloride yielding toremifene base which is converted to toremifene citrate by the addition of citric acid in water/ethanol (Orion, 1996).

1.2.2 *Use*

Toremifene, an antioestrogenic compound and a chlorinated analogue of tamoxifen, has been investigated for the treatment of metastatic breast cancer in postmenopausal women. It has been studied in animal experiments (Kangas *et al.*, 1986) and in clinical phase I (Kivinen & Mäenpää, 1990; Hamm *et al.*, 1991) and phase II (Valavaara *et al.*, 1988; Valavaara & Pyrhönen, 1989; Hietanen *et al.*, 1990; Valavaara, 1990; Jönsson *et al.*, 1991; Pyrhönen *et al.*, 1994) trials (see Glossary, p. 449).

Toremifene is being studied, in comparison with tamoxifen, in at least five different phase III trials (see Glossary, p. 449) in women with metastatic breast cancer. In order to clarify the dose-dependence of its action, daily doses of 60–240 mg are being used, in comparison with 20–40-mg daily doses of tamoxifen (Pyrhönen, 1990). One worldwide three-armed randomized phase III trial of tamoxifen (20 mg daily) versus toremifene (60 mg daily) or toremifene (200 mg daily) in postmenopausal women with metastatic breast cancer showed similar response rates and survival in all three arms (Hayes *et al.*, 1995). Results from other comparative phase III trials will soon be available. Three trials of toremifene as adjuvant therapy are also in progress: two performed by the International Breast Cancer Study Group, and one by the Finnish Breast Cancer Group (Orion, 1996).

Like tamoxifen, toremifene has also been tested for use in several other malignant diseases including endometrial carcinoma (Horvath *et al.*, 1990; Mäenpää *et al.*, 1992a), ovarian carcinoma (Mäenpää *et al.*, 1992b) and melanoma (Kleeberg *et al.*, 1993). Studies in patients with advanced breast cancer show some response to treatment and minimal side-effects. This seems to be similar to tamoxifen and there appears to be major cross-resistance between the two drugs (Jönsson *et al.*, 1991; Nomura *et al.*, 1993; Stenbygaard *et al.*, 1993; Vogel *et al.*, 1993; Pyrhönen *et al.*, 1994). Toremifene has beneficial effects on cardiovascular lipid profiles in postmenopausal women with breast cancer (Gylling *et al.*, 1995).

Thus, although toremifene is not yet registered for use in most countries, it is under extensive investigation for therapy of metastatic breast cancer as well as in the adjuvant setting.

Toremifene was developed in Finland and has been available there since 1990. It has been available in Sweden, Russia and the Ukraine since 1994, and became available in Japan in 1995 (Anon., 1995a; Orion, 1996).

1.3 Occurrence

Toremifene is not known to occur as a natural product.

1.4 Regulations and guidelines

A Health Registration Application for toremifene was filed with the European Union's Committee for Proprietary Medicinal Products in December 1994. A New Drug Application for toremifene was filed with the United States Food and Drug Administration in January 1995 (Anon., 1995b). Toremifene was subsequently recommended for approval by the United States Food and Drug Administration (Anon., 1995a) and the European Union's Committee for Proprietary Medicinal Products in October 1995 (Anon., 1995a). It was approved in February 1996 in the European Union for treatment of hormone-dependent metastatic breast cancer in postmenopausal patients (European Commission, 1996).

2. Studies of Cancer in Humans

No report of carcinogenicity or chemopreventive activity of toremifene in humans has been published.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: In a study that also included tamoxifen, groups of 57, 84 and 75 female Sprague-Dawley [Crl:CD(BR)] rats, six weeks of age, were given 0 (control), 12 and 24 mg/kg bw toremifene citrate (purity, 99%) per day by gastric instillation in 0.5% carboxymethylcellulose on seven days per week for up to 12 months. Nine rats in each group were killed at three and six months. At 12 months, 18 control, 36 low-dose and 10 high-dose rats were killed and, at 15 months, 13, 20 and 13 animals in the respective groups were killed; 8, 10 and 34 respectively died during the experiment. All rats, including those found dead or moribund, were subjected to necropsy; organs examined histopathologically included liver, ovaries, uterus, mammary gland, adrenal glands, tail bone, sternum, brain and pituitary. Weight gain in both groups receiving toremifene citrate was less than that in controls. At three months, the incidence of placental-type glutathione *S*-transferase-positive altered hepatocellular foci was 5/9 (56%) control, 3/9 (33%) low-dose and 1/9 (11%) high-dose rats. No liver tumour was found at 12 or 15 months. At 12

months, the incidence of granulosa-cell tumours of the ovary was 1/34 low-dose and 1/10 high-dose rats compared with 0/17 controls. In rats killed at 15 months, three months after cessation of exposure, no ovarian tumour was found. The incidence of hyperplasia, adenoma or carcinoma in the mammary gland and that of pituitary adenoma or carcinoma were zero in toremifene citrate-treated rats (Hard *et al.*, 1993). [The Working Group noted that exposure was limited to 12 months and that the study was terminated at 15 months.]

In a study that also included tamoxifen, groups of 20 female Sprague-Dawley rats, six weeks of age, were given 0 (control), 12 or 48 (MTD, maximum tolerated dose) mg/kg bw toremifene citrate (purity > 99%) per day by gastric instillation in 0.5% carboxymethylcellulose for up to one year. All surviving animals were observed without further exposure for an additional 13 weeks. Five animals from each group were killed after 26 weeks and 52 weeks of treatment; all surviving rats (8 controls, 9 low-dose and 3 high-dose animals) were killed 65 weeks after the beginning of treatment. Weight gain was reduced in both toremifene citrate-treated groups. No liver tumour was found in animals at interim or terminal kills. Findings in tissues other than the liver were not reported (Hirsimäki *et al.*, 1993). [The Working Group noted the small number of animals, that exposure was of 12 months' duration and that the study was terminated at 65 weeks.]

In a study that also included tamoxifen, groups of 10 female Sprague-Dawley rats, six weeks of age, were given 0, 12 or 48 (MTD) mg/kg bw toremifene citrate (purity > 99%) per day by gastric instillation in 0.5% carboxymethylcellulose for 12 months. Groups of five animals were killed at 12 months or after a further 13 weeks of recovery. No liver tumour occurred in any group (Ahotupa *et al.*, 1994). [The Working Group noted the small number of animals and that the study was terminated at 65 weeks.]

As part of a tumour promotion study that also included tamoxifen, groups of 36–37 female Fischer rats, weighing 130 ± 10 g [age not specified], were subjected to partial hepatectomy and three weeks later exposed to toremifene [purity not specified] in the diet at concentrations of 0 (control), 250, 500 or 750 mg/kg diet (ppm) for 6 or 18 months. All exposures to toremifene suppressed body-weight gain in animals killed at six months, and uterine weights were reduced. At this time, no increase was found in either the number or volume of hepatocellular altered foci identified by staining for the placental form of glutathione *S*-transferase or adenosine triphosphatase, whereas γ-glutamyltranspeptidase-positive foci were increased at all doses (dose-related). One hepatic neoplastic nodule was found in controls compared with none in the treated animals. At the terminal kill at 18 months, there was no increase in liver tumours (see Table 1). No kidney tumour was found (Dragan *et al.*, 1995). [The Working Group noted the small numbers of animals and the short duration.]

Groups of 50 male and 50 female Sprague-Dawley rats, six weeks of age, were given toremifene citrate (purity > 98%) in the diet for two years. The rats received mean daily intakes of 0 (control), 0.12, 1.2, 5.0 or 12 mg/kg bw per day. The concentrations of toremifene in the diet were adjusted to maintain constant dose levels in terms of mg/kg bw per day. Toremifene citrate caused decreases in food consumption and body-weight gain in a dose-dependent manner, with high-dose females having 79% food consumption

and 58% body weight compared with controls and high-dose males having 73% and 40%, respectively. Toremifene citrate reduced mortality, principally in the two highest dose groups. Mortality was 2% in the females receiving 12 mg/kg and 16% in those receiving 5 mg/kg, compared with 66% in controls, and 10% and 8% in the corresponding groups of males compared with 40% in controls. No increase in tumours was found. In females, the incidences of mammary and pituitary tumours were reduced, while, in males, the incidences of pituitary and testicular tumours were reduced (see Table 2) (Karlsson *et al.*, 1996). [The Working Group noted that some of the reduced tumour incidences may have been related to body-weight reduction.]

Table 1. Incidences of liver neoplasms in female Sprague-Dawley rats exposed to toremifene

Exposure (mg/kg diet (ppm))	Neoplastic nodules	Hepatocellular carcinomas
None	16/22	0/22
Toremifene, 250	6/22	0/22
Toremifene, 500	4/21	1/21
Toremifene, 750	15/22	0/22

From Dragan *et al.* (1995)

Table 2. Incidence (%) of certain tumours in Sprague-Dawley rats exposed to toremifene citrate

	Dose (mg/kg bw per day)				
	0	0.12	1.2	5.0	12.0
Females					
Mammary tumours	60	14[a]	4[b]	2[b]	4[b]
Pituitary tumours	86	52[b]	2[b]	2[b]	0[b]
Males					
Pituitary tumours	54	38[b]	4[b]	2[b]	4[b]
Testicular tumours	10	12	0	0	0

From Karlsson *et al.* (1996)
[a] $p < 0.01$
[b] $p < 0.001$

In a study that also included tamoxifen, in a compilation of three experiments reported in the proceedings of a meeting, groups of 38, 62 or 64 female Sprague-Dawley rats [age unspecified] were given daily doses of 3, 12 or 48 mg/kg bw by gastric instillation for up to 52 weeks. No tumours of the uterus were seen (Mäntylä *et al.*, 1996).

3.2 Administration with known carcinogens

Rat: Groups of 5–10 (32 in controls) female Sprague-Dawley rats, 50 ± 2 days of age, were given 12 mg/animal 7,12-dimethylbenz[*a*]anthracene (DMBA) as a single gastric instillation in sesame oil. After six weeks, when mammary tumours had reached about 1 cm in diameter, groups were treated with 0, 0.3, 1.0, 3.0, 7.5, 15.0 or 30.0 mg/kg bw toremifene citrate (purity > 98%) [vehicle not specified] per day by gastric instillation for at least five weeks. The numbers of new tumours per animal were 3.0 ± 2.6 in controls and 1.4 ± 1.2 in the 0.3-mg/kg bw, 0.6 ± 0.7 in the 1.0-mg/kg bw, 0.7 ± 1.1 in the 3.0-mg/kg bw, 1.6 ± 2.0 in the 7.5-mg/kg bw, 1.8 ± 2.4 in the 15.0-mg/kg bw and 0.6 ± 1.4 in the 30.0-mg/kg bw toremifene citrate-treated groups, a significant decrease ($p < 0.005$) at all doses, except 0.3 and 15 mg/kg bw (Kangas *et al.*, 1986).

Groups of 20 Sprague-Dawley rats [females], 50 days of age, were given 20 mg per animal DMBA in peanut oil as a single gastric instillation. Twenty-eight days later, groups were given 50, 200 or 800 μg/animal toremifene citrate in peanut oil daily by gastric instillation for four months. By 100 days after DMBA administration, 75% of rats given DMBA alone had developed mammary tumours, many having multiple tumours, whereas, in the group also receiving toremifene (200 or 800 μg), less than 20% had tumours [percentages derived from graphs] and only single tumours. Cessation of toremifene treatment (200 or 800 μg) after four months led to the development of mammary tumours, so that by 4.5 months about 70% of animals had tumours (Robinson *et al.*, 1988).

Two groups of female Sprague-Dawley rats, 50 days old, received 12 mg/animal DMBA [vehicle not specified] as a single gastric instillation. After seven weeks, the animals were given 0 (five rats with 16 mammary tumours) or 3 (six rats with 25 mammary tumours) mg/kg bw toremifene [purity not specified] per day by gastric instillation for five weeks. During treatment, 32 new mammary tumours appeared in the control group and 16 in the treated group (Huovinen & Collan, 1994).

In a tumour promotion study, in which tamoxifen was also studied, groups of 14–22 female Fischer rats, weighing 130 ± 10 g [age not specified], were subjected to partial hepatectomy and 24 h later were given 10 mg/kg bw *N*-nitrosodiethylamine (NDEA) in trioctanoin as a single gastric instillation. Two weeks later, rats were given either basal diet or diet containing 250, 500 or 750 mg/kg diet (ppm) toremifene for 6 or 18 months. All exposures to toremifene depressed body-weight gain in animals killed at six months and uterine weights were depressed. The number and volume fraction of liver occupied by altered hepatic foci identified by any of the histochemical markers used was increased by toremifene. At six months, the incidences of hepatic neoplastic nodules in all groups were 53, 20, 20 and 43% in controls, low-, mid- and high-dose, respectively. At 18 months, the incidence of liver tumours in all groups approached 100%. Toremifene increased the incidence of hepatocellular carcinomas in the high-dose group (2/17, 2/18, 7/16 and 11/18 in the controls, low-, mid- and high-dose respectively). The incidence of kidney tumours was increased by toremifene at the highest dose (Table 3) (Dragan *et al.*, 1995).

Table 3. Incidences of kidney neoplasms in female Fischer rats exposed to toremifene after NDEA

Exposure (mg/kg diet (ppm))	Renal cell adenomas	Renal cell carcinomas
None	5/19[a]	0/19
Toremifene, 250	0/18	0/18
Toremifene, 500	7/16	2/16[b]
Toremifene, 750	12/20[c]	5/20[c]

From Dragan *et al.* (1995)
[a] [$p = 0.002$; Cochran Armitage test for trend]
[b] [not significant; Fisher's exact test]
[c] [$p = 0.03$; Fisher's exact test]

In a study that also included tamoxifen, groups of virgin female Sprague-Dawley rats aged 43 days were randomized into groups of 20 and allocated to control diet or a diet containing 100 mg/kg diet (ppm) toremifene citrate (reduced to 50 mg/kg at 71 days of age because of reduced body-weight gain). Seven days later, groups were given either 50 mg/kg bw *N*-methyl-*N*-nitrosourea or saline by intravenous injection. Animals were killed when moribund and the experiment was terminated at 180 days. Toremifene citrate reduced the incidence of mammary tumours to 46% compared with 100% in controls ($p < 0.05$); the multiplicity was reduced to 0.7 ± 0.2 from 10.6 ± 1.2 ($p < 0.05$) and latency was increased to 166 ± 8 days from 53 ± 4 days ($p < 0.05$) in the controls (Moon *et al.*, 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

In a study with 70 postmenopausal female volunteers, Anttila *et al.* (1990) found that toremifene was well absorbed and over 99% bound to plasma proteins. Peak serum concentration was usually reached within 4 h and the mean half-life of distribution was generally around 4 h. A further study (Anttila *et al.*, 1995) was conducted on 10 healthy subjects (7 men and 3 women; body weight, 80.9 ± 20.3 kg) given a single oral dose of 120 mg toremifene, following an overnight fast. Measured pharmacokinetic parameters for toremifene, *N*-desmethyltoremifene and deaminohydroxytoremifene in serum, respectively, were: maximum concentrations (C_{max}), 414 ± 173 ng/mL, 130 ± 53 ng/mL and 38 ± 24 ng/mL at median times of 2 h, 72 h and 2 h, the areas under the integrated time × concentration curves (AUC) being 28.4 ± 12.3, 94.1 ± 77.5 and 0.48 ± 0.66 (μg × h/mL).

Elimination half-life ($t_{½}$) values were 6.2 ± 2.2 days for toremifene and 21.0 ± 24.1 days for *N*-desmethyltoremifene. Apparent clearance of toremifene after oral dosing was 5.1 L/h and its apparent volume of distribution was 958 ± 309 L. Multiple dosing with 60 mg toremifene per day resulted in an average steady-state serum level of 800 ng/mL within six weeks after the start of therapy (Anttila *et al.*, 1990). Postmenopausal patients (19 women) receiving high doses of toremifene (240–780 mg/day) for advanced breast cancer showed plasma concentrations ranging from 1.5 to 4.0 μg/mL (Bishop *et al.*, 1992). In 70 postmenopausal patients with advanced breast cancer receiving single oral daily doses of either 10, 20, 40, 60, 200 or 400 mg toremifene for eight weeks, the time to reach steady-state plasma concentrations was between one and five weeks (one to two weeks for the 200 and 400 mg doses). The time to peak concentration was 1.5–4.5 h. The peak toremifene concentrations were 1117–1270 ng/mL (at a dose of 60 mg/day) and 198–669 ng/mL (at a dose of 20 mg/day). Plasma concentrations of 4-hydroxytoremifene were detectable only at high doses (200–400 mg per day) of toremifene, typical peak concentrations being 383–515 ng/mL after a 400 mg-dose. The peak concentrations of *N*-desmethyltoremifene were 538–2622 ng/mL (at a dose of 20 mg/day), 2709–5769 ng/mL (at a dose of 60 mg/day) and 7937–9135 ng/mL (at a dose of 400 mg/day) (Wiebe *et al.*, 1990).

Toremifene undergoes extensive demethylation and hydroxylation to active and inactive metabolites via hepatic mixed function oxidases (see Figure 1). In human urine, four unconjugated and three glucuronide-conjugated metabolites were detected in one study, but only 4-hydroxytoremifene glucuronide was identified (Watanabe *et al.*, 1989). In the study of Anttila *et al.* (1990), *N*-desmethyltoremifene was the major metabolite in serum and was present at a concentration twice that of toremifene; other metabolites included 4-hydroxytoremifene, deaminohydroxytoremifene (one tenth of toremifene concentration (Anttila *et al.*, 1990)) and didesmethyltoremifene (Kangas, 1990).

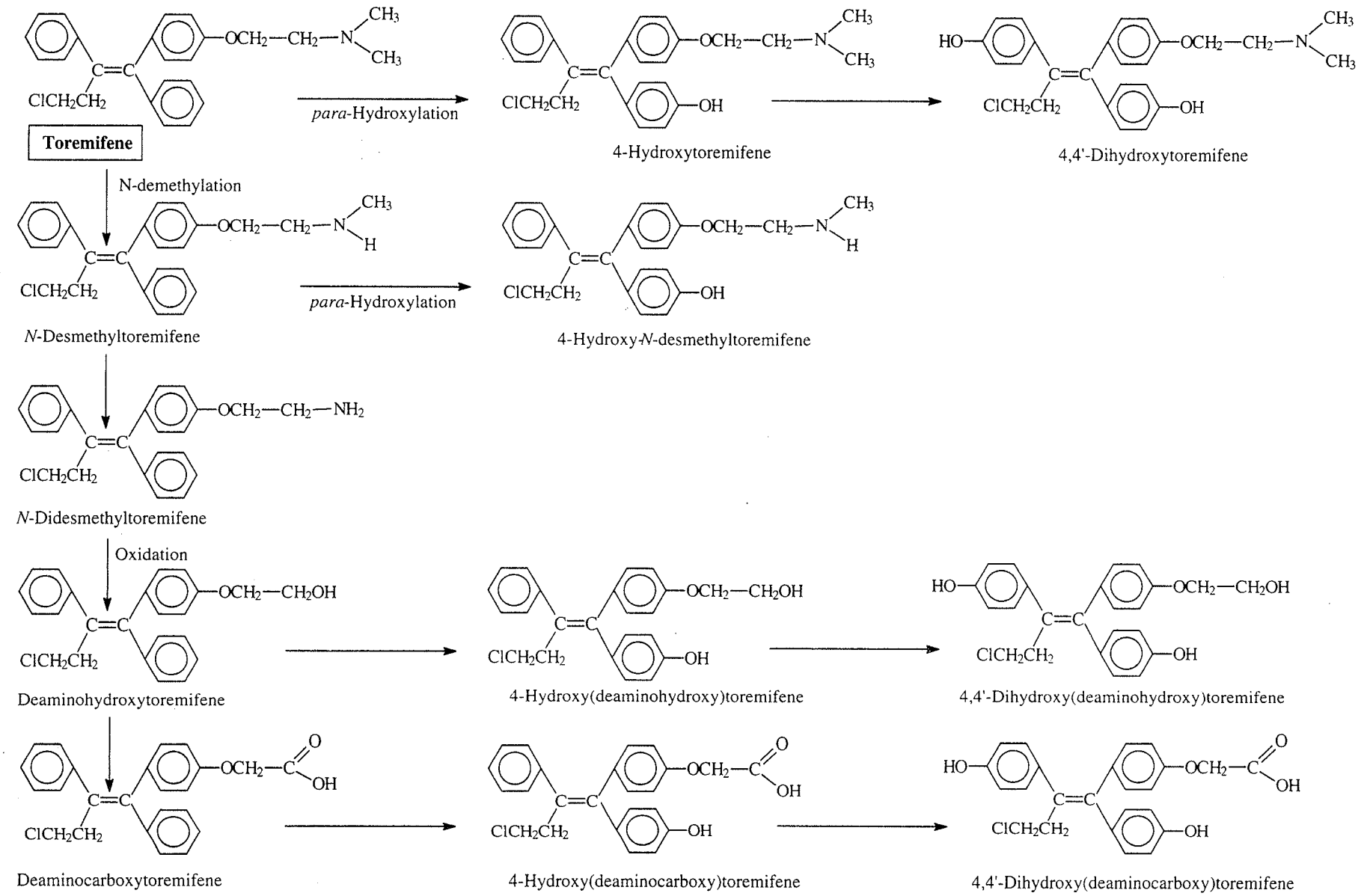
Elimination of toremifene is slow, with a mean half-life of five days (Anttila *et al.*, 1990). The terminal half-lives for elimination of toremifene, *N*-desmethyltoremifene and 4-hydroxytoremifene are five, six and five days, respectively (Wiebe *et al.*, 1990).

Enterohepatic recirculation of toremifene has been reported in humans (Wiebe *et al.*, 1990). The majority of a dose of toremifene is excreted as metabolites in faeces (Anttila *et al.*, 1990) and the long half-life of toremifene may be due to both plasma protein binding and enterohepatic recirculation (Wiebe *et al.*, 1990).

4.1.2 *Experimental systems*

Following administration of [^{3}H]toremifene to female Sprague-Dawley rats by intravenous injection, 70% of the total radioactivity was eliminated within 13 days, with more than 90% of this appearing in the faeces. Toremifene metabolites have been identified in rats and others have been postulated (see Figure 1). 4-Hydroxytoremifene is a major metabolite in rats and is present in urine at twice the level of *N*-desmethyltoremifene (Sipilä *et al.*, 1990). Toremifene is metabolized and excreted by isolated perfused rat liver, but a significant amount binds to the reperfusion circuit. Furthermore, clinically relevant doses of toremifene do not appear to inhibit hepatic mixed function

Figure 1. Postulated metabolic pathways of toremifene



Adapted from Kangas (1990)

oxidase activity, as indicated by elimination of antipyrine (Webster *et al.*, 1993). Enterohepatic recirculation of toremifene has been reported in rats and small amounts of unchanged toremifene have been found in the faeces, suggesting biliary secretion (Sipilä *et al.*, 1990).

The major pathways of toremifene metabolism are mediated mainly by a CYP3A4 enzyme in human liver microsomes (Berthou *et al.*, 1994).

Administration to rats of 0.12 mmol/kg bw toremifene per day for four days by gastric instillation increased the metabolism of benzyloxy- and pentoxyresorufin 10–80-fold, while ethoxyresorufin metabolism hardly changed (White *et al.*, 1993).

4.2 Toxic effects

4.2.1 *Humans*

In a phase I clinical trial of 107 cancer patients (74 with breast cancer), toremifene was administered at doses from 10 to 400 mg per day for eight weeks to groups of 11–26 patients (age range, 25–80 years; mean, 58 years) (Kohler *et al.*, 1990). In general, toremifene was well tolerated at all doses tested. Gastrointestinal complaints were the most common side-effects (at all dose levels). Nausea and vomiting, usually mild, were reported by 43% of patients. Antioestrogenic side-effects included hot flushes (29%), vaginal discharge (8.4%) and vaginal bleeding (2.8%). Other effects observed were related to the central nervous system and included dizziness/vertigo (12%), lethargy/-fatigue (10%), headaches (7%), insomnia (4%), anxiety (3%) and irritability (2%). The only ophthalmological finding was dry eye (reduced tearing), which was reported by three patients at higher doses. There was no change in electrolytes, liver function, renal function or serum lipids. No dose-related change in total leukocytes, granulocytes or platelets was found. A moderate decrease in antithrombin III activity was found. A decline in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels (but only at concentrations equal to or greater than 40 mg/day) and an increase in sex hormone-binding globulin (SHBG) were noted. No change was observed in cortisol, prolactin, oestrone or 17β-oestradiol levels or in thyroid function tests. Similar results were reported from a phase I study in 72 healthy postmenopausal volunteers in which toremifene was given within the dose range of 3–680 mg as a single dose or on five consecutive days (Kivinen & Mäenpää, 1990). Other effects of 60 mg/day toremifene given to breast cancer patients for 3, 6 or 12 months included a stimulatory effect on cell-mediated immunity, according to a positive effect on mitogen-stimulation tests (Valavaara *et al.*, 1990) [The Working Group noted that the control group consisted of healthy women.]

Thirty-one gynaecologically asymptomatic postmenopausal breast cancer patients with intact uteri were randomized to receive 20 mg tamoxifen or 60 mg toremifene as adjuvant treatment (Tomás *et al.*, 1995). Pap smear, endometrial biopsy, hysteroscopy and curettage were performed before treatment and at the end of 6 and 12 months of treatment. In the toremifene group, endometrial thickness increased from 3.9 mm before treatment to 6.0 mm at six months and 7.0 mm at 12 months. Proliferation in endometrial

cytology in the toremifene group at the three observation times, respectively, occurred in 0/13, 3/11 and 2/10 patients. There was no significant difference between toremifene and tamoxifen in any of the parameters investigated.

A three-armed randomized comparison was performed with toremifene at 60 mg/day (n = 221) and 200 mg/day (n = 212) and tamoxifen at 20 mg/day (n = 215) in postmenopausal patients with hormone receptor-positive or unknown metastatic breast cancer (Hayes *et al.*, 1995). The group receiving 200 mg/day toremifene experienced significantly more nausea (p = 0.027), but no other significant difference in toxicity or quality of life was reported among the three arms of the trial. Clinical tumour flare (a transient increase in bone and/or musculoskeletal pain within two weeks of starting the drug) occurred in 16% of the 60-mg toremifene and 19% of the 200-mg toremifene groups (and 19% of the tamoxifen group). Seventeen patients died during the study or within 30 days of the last dose due to causes believed not to be secondary to metastatic breast disease. These were similarly distributed, with no significant differences among the three arms (nine and six in the 60-mg and 200-mg toremifene groups, respectively, and two in the tamoxifen group). Serious but non-lethal adverse events in the 60-mg and 200-mg toremifene and tamoxifen groups, respectively, included pulmonary embolism (5, 2, 2), cerebrovascular accidents (0, 3, 0), thrombosis (1, 0, 1), impaired liver function texts (alanine transaminase ≥ 100 IU/L, 11, 22, 4; total bilirubin ≥ 2 mg/dL, 3, 7, 4) and corneal keratopathies (4, 8, 2). Twenty-one patients withdrew from the study because of toxicity; 6 and 12 in the 60-mg and 200-mg toremifene groups, respectively, and 3 in the tamoxifen group.

4.2.2 *Experimental systems*

In a preliminary toxicity study in which toremifene was administered to rats at doses of up to 48 mg/kg bw for 26 weeks, no ocular or hepatic changes were observed (Kangas *et al.*, 1986). [No further details were given.]

Toremifene at a steady-state concentration of 10 μg/mL (a high, but clinically relevant concentration) caused a significant decrease in bile flow in isolated perfused rat liver and therefore appears to impair liver function, but it did not have any effect on antipyrine elimination (Webster *et al.*, 1993).

In Sprague-Dawley rats given 250–750 mg/kg of diet (ppm) toremifene for 18 months, uterine weights were depressed (Dragan *et al.*, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In a study reported only as an abstract, Hirsimäki *et al.* (1990) stated that pregnancy was not compromised in rats given oral doses of up to 50 mg/kg bw toremifene per day, although toxic effects occurred in the dam at doses of 10 mg/kg bw per day or higher. In

rabbits, abortion occurred with doses of 10 mg/kg bw per day and total litter loss with 50 mg/kg bw per day. Fertility of male rats was reduced after 10 weeks of oral treatment with 25 mg/kg bw per day, but not at lower levels. This reduction in fertility was accompanied by a reduction in the weight of the reproductive organs. Females became acyclic when treated with doses of 0.2 mg/kg bw per day or higher and, although they mated, they did not become pregnant. At a dose of 0.04 mg/kg bw per day, the oestrus cycle was normal, and mating and pregnancy were successful. Oestrus cycles recovered within two weeks and mating occurred, but treatment could not be recommenced until day 6 of gestation, otherwise implantation was prevented. At doses of 1 mg/kg bw per day or more, parturition difficulties occurred.

4.4 Genetic and related effects

4.4.1 *Humans*

No studies were available to the Working Group.

4.4.2 *Experimental systems*

Mutagenicity (see also Table 4 for references and Appendices 1 and 2)

Toremifene induced micronucleus formation in MCL-5 cells, a genetically engineered human lymphoblastoid cell line that expresses native CYP1A1 and transfected CYP1A2, CYP2A6, CYP2E1 and CYP3A4 and epoxide hydrolase, and also weakly in similar cell lines that expressed CYP2E1 or 3A4 but not in a line expressing CYP2D6.

DNA adducts in vivo

A very low level of DNA adducts (0.85 ± 0.1 adducts per 10^8 nucleotides) was detected by ^{32}P-postlabelling in the livers of female Fischer 344 rats given 0.12 mmol/kg toremifene per day by gastric instillation for four days and killed 24 h after the final treatment (White *et al.*, 1992).

Treatment of female Sprague-Dawley rats with up to 90 µmol/kg toremifene per day by gastric instillation for 10 days did not lead to formation of detectable DNA adducts in the liver (Montandon & Williams, 1994). Similarly, DNA adducts were not detected in the livers of Crl:CD(BR) rats (Sprague-Dawley) given 48 mg/kg toremifene (0.12 mmol/kg) per day by gastric instillation for seven days and killed 24 h later (Hard *et al.*, 1993).

A very low level of DNA adducts (0.02 adducts per 10^8 nucleotides) was detected by ^{32}P-postlabelling in cultured human lymphocytes treated with 100 µg/mL toremifene (Hemminki *et al.*, 1995). Adducts were not detected at lower concentrations.

A low level of microsome-mediated DNA adduct formation by toremifene was detected by ^{32}P-postlabelling (Hemminki *et al.*, 1995). In the presence of NADP and glucose-6-phosphate, toremifene concentrations of 1 mM gave adduct levels of 0.04 adducts per 10^8 nucleotides with rat microsomes and 0.012 adducts per 10^8 nucleotides with human microsomes.

Table 4. Genetic and related effects of toremifene

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MIH, Micronucleus test, human lymphoblastoid MCL-5 cells *in vitro*	+[c]	NT	0.5	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h1A1 cells expressing CYP1A1 *in vitro*	–	NT	1.5	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h1A2 cells expressing CYP1A2 *in vitro*	–	NT	1.5	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h2E1 cells expressing CYP2E1 *in vitro*	(+)	NT	0.75	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h3A4 cells expressing CYP3A4 *in vitro*	(+)	NT	0.75	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h2D6 cells expressing CYP2D6 *in vitro*	–	NT	1.5	Styles *et al.* (1994)
BID, Binding (covalent) to DNA, human lymphocytes *in vitro*	(+)	NT	100	Hemminki *et al.* (1995)
BVD, Binding (covalent) to DNA, female Fischer 344/N rat liver *in vivo* (^{32}P-postlabelling)	(+)		50 po × 4	White *et al.* (1992)
BVD, Binding (covalent) to DNA, female SD rat liver *in vivo* (^{32}P-postlabelling)	–		48 po × 7	Hard *et al.* (1993)
BVD, Binding (covalent) to DNA, female SD rat liver *in vivo* (^{32}P-postlabelling)	–		33 po × 10	Montandon & Williams (1994)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day

[c] Expressing native CYP1A1 and transfected CYPs 1A2, 2A6, 3A4 and 2E1 and epoxide hydrolase; cytochalasin B-arrested

Horseradish peroxidase activated toremifene to a reactive intermediate which bound covalently to both DNA and protein (Davies *et al.*, 1995).

4.5 Hormonal effects

4.5.1 *Humans*

The effects of toremifene on endocrine parameters in healthy postmenopausal women and in women with advanced breast cancer have been studied. Oestrogenic effects were evaluated by measuring concentrations of LH, FSH, SHBG and prolactin, and antioestrogenicity in 17β-oestradiol-primed postmenopausal women by vaginal cornification index. During eight weeks of treatment with 60 or 200 mg/day toremifene, FSH levels decreased by a mean of 29 and 53%, respectively, and LH levels by approximately 20 and 50%. SHBG concentrations increased about two-fold at both dose levels (Hamm *et al.*, 1991). Prolactin concentrations in serum did not change, although, in patients with basal prolactin, a decrease to the normal level was seen (Számel *et al.*, 1994). The no-effect dose level of toremifene in the vaginal cornification antioestrogenic assay was 10 mg daily. There was no clear dose–response relationship of the vaginal cornification index at doses of 20–200 mg/day (Hamm *et al.*, 1991).

4.5.2 *Experimental systems*

Toremifene bound to oestrogen receptors in rat uterus competitively with [^{3}H]17β-oestradiol, the IC_{50} concentration being 0.5 μmol/L and the dissociation constant 1 nM (Kallio *et al.*, 1986; Simberg *et al.*, 1990). It induced binding of oestrogen receptors to the nuclear compartment and increased progesterone receptor concentrations in the rat uterus *in vivo* during five days of administration (Kallio *et al.*, 1986).

The oestrogenic and antioestrogenic actions of toremifene were studied by a uterotrophic assay in immature and ovariectomized adult mice and rats given toremifene daily for three days (oestrogenicity) and together with 17β-oestradiol (antioestrogenicity). Significant oestrogenic and antioestrogenic responses were observed in mice at the lowest test dose reported, 0.05 mg/kg. In rats, the lowest dose inducing a significant oestrogenic response was 0.1 mg/kg, while an antioestrogenic response was observed at 0.01 mg/kg (Kallio *et al.*, 1986; Kangas, 1990; di Salle *et al.*, 1990).

The hormonal effects of toremifene were studied in rat liver by measurement of cytosolic (reduction) and nuclear (increase) oestrogen receptors. Toremifene produced an oestrogen agonistic effect (Kendall & Rose, 1992).

Triphenylethylene antioestrogens have tissue- and species-specific hormonal effects. Toremifene is predominantly oestrogenic in mice, antioestrogenic in rats and humans (Kangas, 1992) and both oestrogenic and antioestrogenic in monkeys (Wood *et al.*, 1992).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Toremifene, a chlorinated analogue of tamoxifen, was first marketed in 1990 and by 1995 was registered in five countries. It is currently undergoing further clinical trials for the treatment of metastatic breast cancer as well as trials for use as adjuvant therapy.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Toremifene was tested for carcinogenicity in one study by oral administration to male and female rats and in four studies of limited duration in female rats. No increase in tumour incidence was observed in these studies. In the one study of long duration, toremifene decreased the incidence of tumours in some hormone-dependent tissues, notably mammary gland.

In one study in female rats, toremifene increased the incidence of kidney tumours and the proportion of malignant liver tumours induced by *N*-nitrosodiethylamine.

In four other experiments in rats, toremifene inhibited the development of 7,12-dimethylbenz[*a*]anthracene- or *N*-methyl-*N*-nitrosourea-induced mammary tumours.

5.4 Other relevant data

Toremifene is well absorbed in humans. The major metabolites result from *N*-demethylation, hydroxylation and deamination, and are excreted predominantly in faeces. The elimination half-life is about six days. The metabolism is qualitatively similar, but quantitatively different, in rats.

In a single study, no teratogenic effect of toremifene was found in rats.

Toremifene induced micronucleus formation in one study that used genetically engineered cell lines. Low levels of DNA adducts were detected in rat liver in one of three studies. Low levels of DNA adduct formation have also been reported in human lymphocytes *in vitro*.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of toremifene.

There is *inadequate evidence* in experimental animals for the carcinogenicity of toremifene.

Overall evaluation

Toremifene is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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[1]For definition of the italicized terms, see Preamble, pp. 22–25.

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